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BIOLOGICAL RESURFACING OF FULL-THICKNESS DEFECTS IN PATELLAR ARTICULAR CARTILAGE OF THE RABBIT

INVESTIGATION OF AUTOGENOUS PERIOSTEAL GRAFTS SUBJECTED TO CONTINUOUS PASSIVE MOTION

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We compared the effects of continuous passive motion with those of intermittent active motion on the results of the resurfacing with autogenous periosteal grafts of full-thickness defects on the articular surface of rabbit patellae.

Of 45 rabbits with defects, 30 received grafts. Fifteen of these had continuous passive motion for two weeks and intermittent active motion for four weeks; the other 15 had intermittent active motion for six weeks. In 15 the defects were not grafted (control group) and they had intermittent active motion for six weeks. Ten more rabbits had a sham operation. Six weeks after surgery, the results were assessed by the gross appearance, histology, histochemistry, immunohistochemistry and electron microscopy.

By all assessments the quality of neochondrogenesis produced by periosteal grafts was superior to that in ungrafted defects ($p < 0.05$) and the results in continuous passive motion treated animals were superior to those in intermittent active motion treated animals ($p < 0.05$). The periosteal grafts produced hyaline cartilage containing type II collagen but the organisation of its fibres was irregular.

The articular cartilage of the patella is the thickest in the human body and the most frequent site of degeneration which may lead to osteoarthritis of the patellofemoral joint (Insall, Falvo and Wise 1976). The causes of patellofemoral pain include malalignment, recurrent subluxation or dislocation, articular fractures and osteoarthritis, but the most common, especially in adolescents and young adults, is chondromalacia patellae (Bentley and Dowd 1984). The non-operative treatment of this is often disappointing (Ogilvie-Harris and Jackson 1984).

Diseased or damaged articular cartilage has an extremely limited potential for either healing or regeneration (Salter et al 1980). We have recently shown that partial-thickness chondral shaving in the rabbit not only fails to stimulate regeneration but also leads to progressive

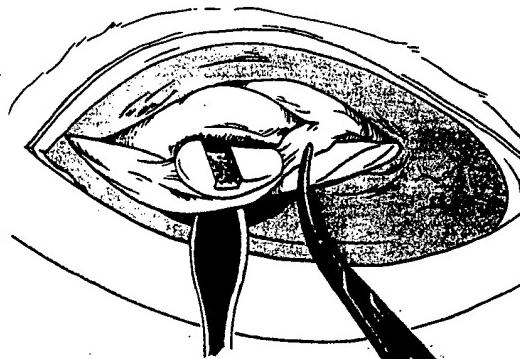


Fig. 1

The experimental model. The patella is everted to show the defect in the articular surface, which extends down into cancellous bone.

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degeneration of the underlying cartilage, whereas full-thickness subchondral abrasion does have the potential to stimulate such regeneration, especially under the influence of continuous passive motion (CPM) (Kim, Moran and Salter 1991).

We have also shown the chondrogenic potential of free autogenous periosteal grafts stimulated by CPM for four weeks in resurfacing full-thickness cartilage defects in rabbits (O'Driscoll, Keeley and Salter 1986; O'Driscoll

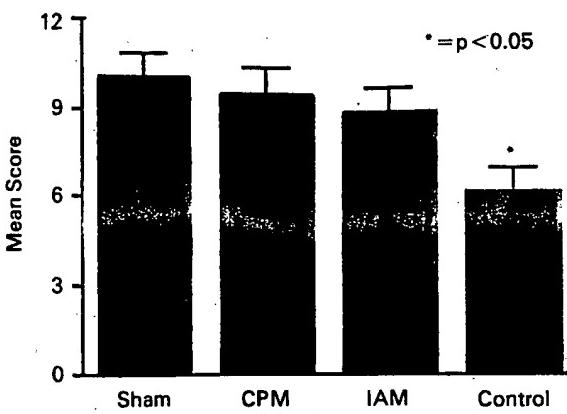
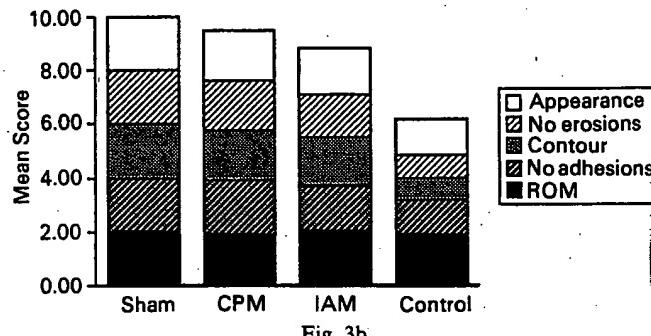


Fig. 3a. Mean total score for gross appearance in each group (bar = SD).



Results for gross appearance giving mean scores for each characteristic (see Table III).

Table III. Results for gross appearance

Characteristic	Group*						
	CPM (n=15)	IAM (n=15)	Control (n=15)	n	%	n	%
Range of motion							
Equal to opposite limb	13	87	15	100	13	87	
50% to 100% of opposite limb	2	13	0	0	2	13	
<50% of opposite limb	0	0	0	0	0	0	
Mean	1.87		2.00		1.87		
Intra-articular adhesions							
None	15	100	12	80	8	53	
Minimal (fine, loose fibrous tissue)	0	0	2	13	4	27	
Major (thick, dense fibrous tissue)	0	0	1	7	3	20	
Mean	2.00		1.73		1.33		
Restoration of articular surface contour							
Complete	14	93	12	80	3	20	
Partial	0	0	2	13	6	40	
None	1	7	1	7	6	40	
Mean	1.87		1.73		0.80		
Erosion of cartilage							
None	13	87	10	66	4	27	
Graft only	2	13	4	27	4	27	
Graft and adjacent normal cartilage	0	0	1	7	7	46	
Mean	1.87		1.60		0.80		
Appearance of cartilage							
Translucent	13	87	11	73	8	53	
Opaque	2	13	4	27	4	27	
Discoloured or irregular	0	0	0	0	3	20	
Mean	1.87		1.73		1.33		

* CPM, continuous passive motion; IAM, intermittent active motion

Table IV. Histological and histochemical results

Characteristic	Group*						
	CPM (n=15)	IAM (n=15)	Control (n=15)	n	%	n	%
Nature of predominant tissue							
Cellular morphology							
Hyaline articular cartilage	13	87	9	60	5	33	
Incompletely differentiated	2	13	3	20	4	27	
Fibrous tissue or bone	0	0	3	20	6	40	
Mean	3.6		2.6		1.6		
Safranin-O staining of the matrix							
Normal or near normal	8	53	5	33	2	13	
Moderate	7	47	4	27	4	27	
Slight	0	0	6	40	9	60	
Mean	2.6		1.6		0.8		
Structural characteristics							
Surface regularity							
Smooth and intact	10	67	9	60	0	0	
Superficial horizontal lamination	3	20	3	20	2	13	
Fissures, 25% to 100% of thickness	2	13	2	13	5	33	
Severe disruption, or fibrillation	0	0	1	7	8	54	
Mean	2.5		2.3		0.6		
Structural integrity							
Normal	12	80	9	60	0	0	
Slight disruption, including cysts	3	20	3	20	6	40	
Severe disintegration	0	0	3	20	9	60	
Mean	1.8		1.4		0.4		
Thickness							
100% of normal adjacent cartilage	15	100	13	86	0	0	
50% to 100% of normal cartilage	0	0	1	7	3	20	
0% to 50% of normal cartilage	0	0	1	7	12	80	
Mean	2.0		1.8		0.2		
Bonding to adjacent cartilage							
Bonded at both ends of graft	7	47	3	20	0	0	
Bonded at one end, or partially at both ends	6	40	6	40	6	40	
Not bonded	2	13	6	40	9	60	
Mean	1.3		0.8		0.4		
Freedom from cellular changes of degeneration							
Hypocellularity							
None	8	53	7	47	0	0	
Slight	3	20	3	20	2	13	
Moderate	3	20	3	20	3	20	
Severe	1	7	2	13	10	67	
Mean	2.2		2.0		0.5		
Chondrocyte clustering							
None	10	67	7	48	0	0	
<25% of cells	4	26	4	26	5	33	
25% to 100% of cells	1	7	4	26	10	67	
Mean	1.6		1.2		0.3		
Freedom from degenerative changes in adjacent cartilage							
Normal cellularity, no clusters, normal staining	13	86	7	47	4	27	
Normal cellularity, mild clusters, moderate staining	1	7	3	20	5	33	
Mild or moderate hypocellularity, slight staining	1	7	3	20	5	33	
Severe hypocellularity, poor or no staining	0	0	2	13	1	7	
Mean	2.8		2.0		1.8		

* CPM, continuous passive motion; IAM, intermittent active motion

microscopy were removed from the knee and immediately cut with a scalpel into small pieces (2 x 2 mm) and fixed for 24 hours in 2.5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.3). They were then fixed with 2% osmic acid, dehydrated through a graded series of ethanol and propylene oxide solutions, and embedded in Epon 812.

Thick sections (10 to 20 µm) were stained with toluidine blue and used to screen for major changes by light microscopy. Suitable areas of the sections were cut



Fig. 4a

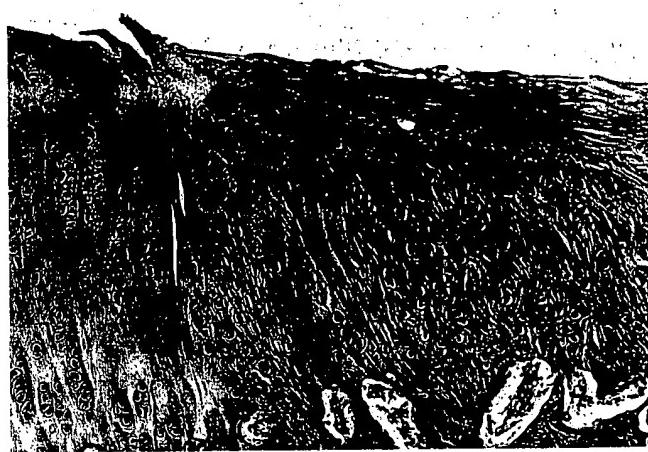


Fig. 4b

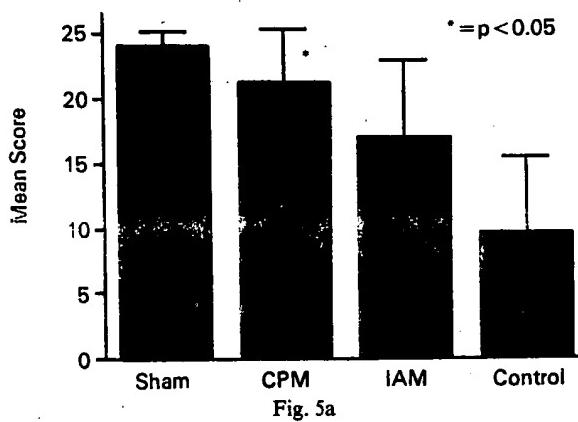


Fig. 4c

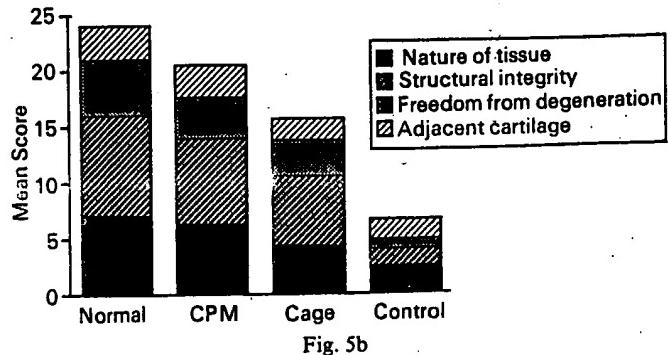


Fig. 4d

Typical histological appearances at six weeks after surgery. Figure 4a – Sham operated. There is deep staining of the glycosaminoglycan matrix, a smooth intact articular surface, even distribution and columnar arrangement of the chondrocytes, and lack of nests or clones of chondrocytes (safranin-O $\times 65$). Figure 4b – A CPM-treated patella, showing the intensity of staining of the repair tissue matrix and the smooth intact surface, well bonded to the adjacent normal cartilage. The cells are orientated in columns and are evenly distributed. There are only occasional chondrocyte clones and the adjacent normal cartilage shows slightly decreased staining (safranin-O $\times 65$). Figure 4c – An IAM-treated patella. The surface of the repair tissue is less smooth and less well bonded to the adjacent cartilage than in CPM-treated animals. The cells are more disorganized and are not grouped into discrete columns. More chondrocyte clones are present (safranin-O $\times 65$). Figure 4d – Control patella, showing poor staining of the repair tissue, and an irregular surface, poorly bonded to the adjacent cartilage, which has significant damage. Many of the cells appear to be more fibroblastic and have no distinct order or distribution. Many chondrocyte clones are present (safranin-O $\times 65$).



Mean total histology score for each group.



Mean histology scores for each characteristic in each group (see Table IV).

into ultra-thin sections with a microtome (Porter-Blum MT2, Newton, Connecticut). After double staining with uranyl acetate and lead citrate, they were examined by electron microscopy (Hitachi H-300, Hitachi Ibargi, Japan).

Statistics. Gross and histological results were analysed using the Kruskal-Wallis test, a non-parametric one-way analysis of variance, necessary for categorical data. The immunohistochemical specimens were rank-ordered with respect to the intensity and distribution of the staining compared with the normal specimens, and evaluated by the Wilcoxon rank-sum test for non-parametric data. A p value < 0.05 was considered to be statistically significant.

Transmission electron-microscopic appearances were assessed qualitatively to determine if the cells and matrix present resembled those found in the patellar articular cartilage of normal rabbits. Only two specimens were examined from each group, and therefore detailed statistical analysis was not performed.

RESULTS

One animal died from anaesthetic complications and three from Pasteurella pneumonia. Two were excluded because of septic arthritis. These six animals were replaced.

Gross appearance. The typical gross appearance for each of the four treatment groups is shown in Figure 2 and the scores are summarised in Table III and Figure 3. Minor flexion contractures averaging 10° were found in 13% of the CPM and control animals but in none of the sham or IAM groups ($p < 0.05$). Intra-articular adhesions were seen in 13% of the IAM and 33% of the control animals but in none of the CPM or sham groups ($p < 0.05$).

Restoration of articular surface contour was complete in 86% of the CPM group, 73% of the IAM group, and only 6% of the control group ($p < 0.01$). The cartilage showed normal translucency in all of the sham group, 87% of the CPM group, 20% of the IAM group, and in none of the control group ($p < 0.01$). Erosions of the cartilage around the defect or of the femoral groove were found in all the animals in the control group, 40% of the IAM group, 13% of the CPM group, and none of the sham group ($p < 0.05$).

Histological and histochemical analysis. The typical histological characteristics of the newly-formed tissue for each treatment group are shown in Figure 4, and are scored in Table IV and Figure 5.

Immunohistochemistry. With monoclonal antibody methods, rabbit articular cartilage stains positively for type II collagen throughout the matrix, but slightly more at the surface and in the deepest layers (Fig. 6). The transitional zone is slightly less darkly stained, perhaps because of the higher concentration of proteoglycans rather than a decrease in the amount of type II collagen. The thin pericellular zone of matrix around individual

chondrocytes stains only faintly while the territorial matrix immediately surrounding it stains slightly darker than the remaining interterritorial matrix. These slight variations in intensity of staining give a 'halo' effect around the cells. There is no detectable intracellular staining of the chondrocytes, indicating that the antibody is recognising an epitope on the collagen moiety only after its final extracellular processing.

The intensity of the anti-type II collagen staining in the treatment groups approached that of the normal or sham specimens. The CPM group ranked first, followed by the IAM and control groups ($p < 0.05$). In most specimens there was no difference between the intensity of positive staining in the neocartilage and the adjacent normal cartilage.

The distribution of the anti-type II collagen staining, however, was abnormal in all three treatment groups: clumped and swirling areas of intense staining were intermixed with areas of complete absence of stain (Fig. 7). We found no significant difference between the

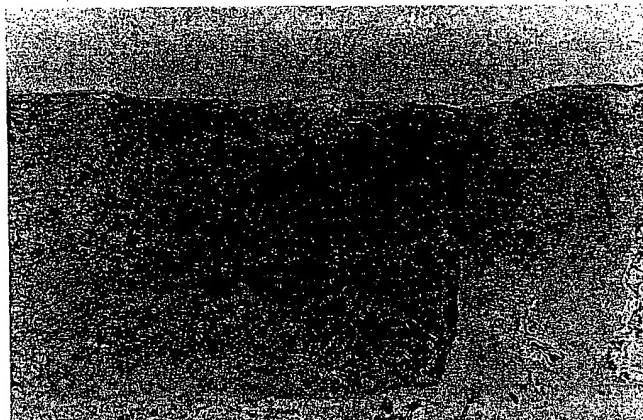


Fig. 6

Specimen from CPM-treated animal stained with anti-type II collagen antibody. The normal articular cartilage is on the right. Note the slightly darker staining at the surface of the cartilage and the decreased staining in the pericellular areas ($\times 26$).

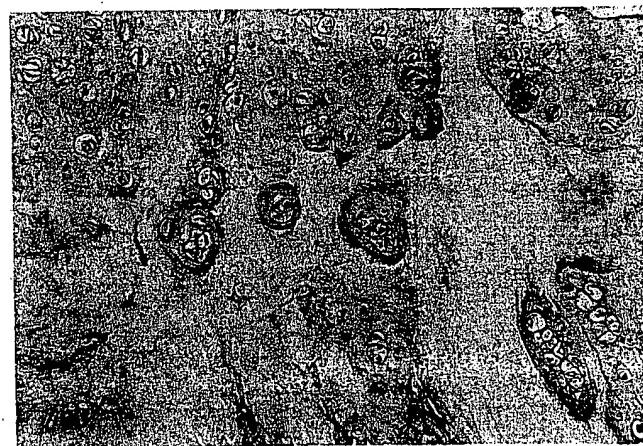


Fig. 7

High-power view of the repair tissue in a CPM-treated animal. Note the uneven distribution of type II collagen staining ($\times 163$).

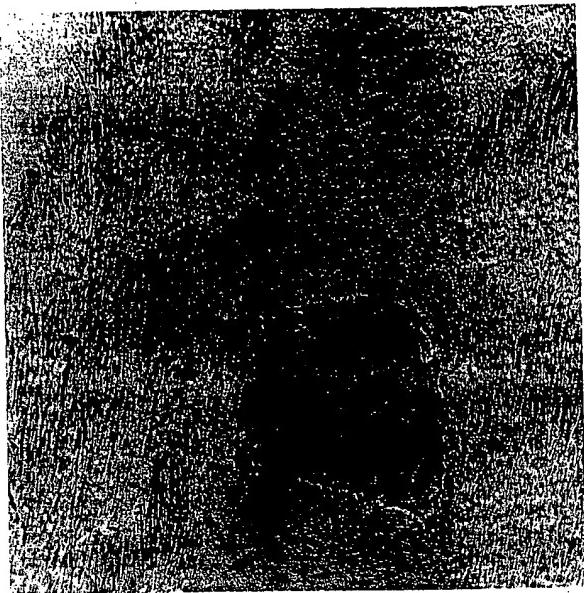


Fig. 8

Normal rabbit articular cartilage shows a homogeneous matrix, with collagen fibres and cells orientated perpendicularly to the joint surface. The cells appear to be metabolically active with abundant mitochondria, rough endoplasmic reticulum, and secretory vesicles ($\times 5749$).



Fig. 9

The typical transmission electron-microscopic appearance of repair tissue from the control group. There is a disorganized appearance, with no apparent organisation of the matrix and cells that resemble fibroblasts rather than chondrocytes ($\times 7316$).

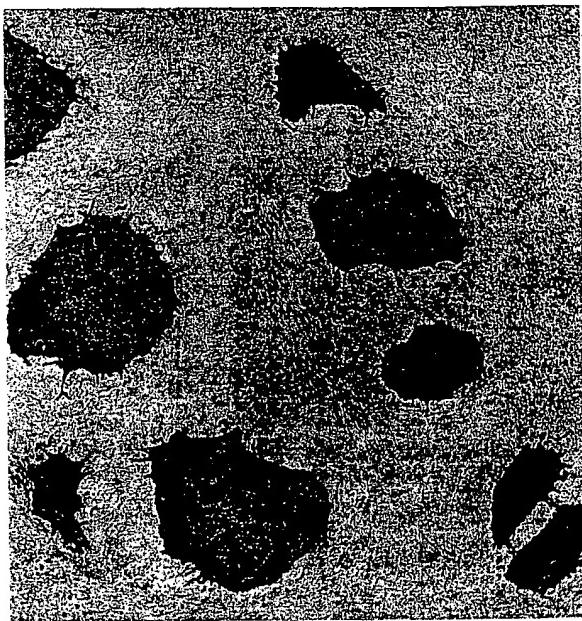


Fig. 10

Typical transmission electron-microscopic appearance of repair tissue from the CPM-treated group. The matrix contains some debris but the collagen fibre cells are orientated similarly to those in normal cartilage. The cells appear to be metabolically active, with large amounts of rough endoplasmic reticulum, mitochondria, and secretory vesicles ($\times 4508$).



Fig. 11

Typical transmission electron-microscopic appearance of repair tissue from the IAM-treated group. The matrix has a less organised appearance with collagen fibres orientated in several planes and a large amount of debris. The cells show metabolic activity but are randomly distributed throughout the matrix, and some appear to be pyknotic or degenerate ($\times 4442$).

treatment group, on the Wilcoxon rank-sum test, but the CPM group ranked slightly higher than the IAM group. The control group ranked lowest ($p = 0.09$).

Transmission electron microscopy. Under the electron microscope, normal rabbit articular cartilage (Davies et al 1962) and cartilage from the sham-operated group showed tangential collagen fibres in the superficial zone blending evenly with the three-dimensional array of fibres in the transitional zone (Fig. 8). In the deepest or radial zone, the collagen fibres were primarily vertical, perpendicular to the joint surface. Throughout all three zones, the fibres were evenly distributed and small in diameter, and thus characteristic of type II collagen. The cells of the superficial zone appeared flattened, lying horizontal to the joint surface. There was little rough endoplasmic reticulum and only small, poorly-developed Golgi complexes, indicating that these cells were not synthetically active. In the transitional zone, the cells were rounded and had abundant rough endoplasmic reticulum and well-developed Golgi complexes. They appeared to be synthetically active, with both lysosomes and secretory vesicles; occasional lipid droplets were also seen in the cytoplasm. The cells of the deep zone were similar in appearance, with reduced amounts of rough endoplasmic reticulum, probably indicating lower synthetic activity.

In the control group (Fig. 9) the operated area revealed a disorganised fibrovascular array consistent with granulation tissue, with only occasional chondroid-like cells. By contrast, the tissue from the operated area in the CPM (Fig. 10) and IAM (Fig. 11) animals showed chondroid tissue almost exclusively. Compared with normal cartilage, this neocartilage had a less organised arrangement of collagen fibrils, with an increased percentage of ground substance, and no stratification into superficial, transitional, and deep zones. The fibrils were usually arranged in bundles or fascicles that tended to be perpendicular to the joint surface. Vertical fissures were occasionally seen and were more common in the IAM group, always showing a thick border of collagen fibres parallel to the margins of the fissure. It is not known whether this was a cause or effect of the fissures. The size of the fibrils also varied: most were of small calibre consistent with type II collagen, but occasional large-diameter fibrils of unknown type were present in both the CPM and IAM specimens.

Fewer chondrocytes were present in the neocartilage, and they were often in scattered clusters. This clustering was more common in the IAM than the CPM group. Occasionally, in CPM specimens the chondrocytes were stacked on each other, resembling physisal chondrocytes (Fig. 10). There was some evidence of chondrocyte degeneration in both groups: there were occasional ghost cells and empty lacunae, with numerous electron-dense matrix vesicles, presumed to be cellular remnants.

In both CPM and IAM groups the individual cells were rounder than normal chondrocytes and appeared to

be more active, with abundant rough endoplasmic reticulum and well-developed Golgi complexes. Perinuclear filaments which are common in normal chondrocytes, were rarely seen. Lipid droplets were more widespread within the cytoplasm of the cells, along with lysosomes and secretory vesicles. Only rarely were there any spindle-shaped fibroblastic cells, and they were found in the deepest layers of the graft, possibly representing residual cells from the fibrous layer of the periosteal graft.

DISCUSSION

Six weeks after surgery, biological resurfacing by a periosteal graft in the CPM and IAM groups was of superior quality to that in the control group. The addition of two weeks of CPM after surgery was shown to improve every aspect of healing and resurfacing of the defect. Even in this group, however, the neocartilage was abnormal in the arrangement and distribution of type II collagen, and in the ultrastructure of the extracellular matrix.

The human patella supports approximately three to four times the body-weight during normal activities. Although there are no comparable data for the rabbit, it is likely that the increased knee flexion produces forces of several times that amount (Maquet 1976). This may explain the poor results in the groups which were allowed immediate full weight-bearing (IAM and control); the higher contact pressures may have been partly responsible.

Nevertheless, the results, especially in the CPM group, are sufficiently encouraging to warrant further investigation including longer-term assessment. If even better results can be achieved, particularly in the microscopic organisation of the collagen framework of the neocartilage, and if these results prove to be durable in the rabbit, then it will be justifiable to start carefully controlled clinical trials. There is some hope of providing a more acceptable outcome in patients severely affected with patellar cartilage disease or damage for which current modes of treatment have little to offer.

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